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Metabolic Engineering of Saccharomyces Cerevisiae for the Production of n-Butanol

Background

Increasing energy costs and environmental concerns have motivated engineering microbes for the production of "second generation" biofuels that have better properties than ethanol. .

Results & Conclusions

Saccharomyces cerevisiae was engineered with an n-butanol biosynthetic pathway, in which isozymes from a number of different organisms (S. cerevisiae, Escherichia coli, Clostridium beijerinckii, and Ralstonia eutropha) were substituted for the Clostridial enzymes and their effect on n-butanol production was compared. By choosing the appropriate isozymes, we were able to improve production of n-butanol ten-fold to 2.5 mg/L. The most productive strains harbored the *C. beijerinckii* 3-hydroxybutyryl-CoA dehydrogenase, which uses NADH as a co-factor, rather than the *R. eutropha* isozyme, which uses NADPH, and the acetoacetyl-CoA transferase from *S. cerevisiae* or *E. coli* rather than that from *R. eutropha*. Surprisingly, expression of the genes encoding the butyryl-CoA dehydrogenase from *C. beijerinckii* (*bcd* and *etfAB*) did not improve butanol production significantly as previously reported in *E. coli.* Using metabolite analysis, we were able to determine which steps in the n-butanol biosynthetic pathway were the most problematic and ripe for future improvement.